Bulletin of the World Health Organization, 55 (4): 441-444 (1977)

Isolation of an arenavirus closely related to Lassa virus from *Mastomys natalensis* in south-east Africa

HERTA WULFF,[1] BRUCE M. McINTOSH,[2] DALE B. HAMNER,[3] & KARL M. JOHNSON [4]

Five unidentified virus strains were recovered from the multimammate mouse, Mastomys natalensis, *during the course of studies on arbovirus infections in Mozambique. These agents were found to be morphologically and immunologically related to Lassa virus. Four of 19 sera from* Mastomys *captured in the study area had antibodies to both Lassa virus and one of the unidentified strains. Although not definitive, the differences noted in results of complement fixation and indirect immunofluorescent tests suggest that these viruses from south-east Africa are not identical to West African Lassa virus.*

During studies on arbovirus infections in Mopeia, central Mozambique, five virus strains that showed no immunological relationship to any arbovirus previously recognized in southern Africa were isolated from *M. natalensis* by intracerebral inoculation of suckling mice. This report summarizes preliminary work, which demonstrates that these agents are immunologically related to, but probably not identical with, Lassa virus of West Africa.

MATERIALS AND METHODS

Virus isolation and propagation

Suckling mice. A 10% suspension of each rodent-tissue pool, consisting of portions of liver, spleen, kidney, lung, and heart, was prepared in phosphate-buffered saline, pH 7.2, containing 0.75% bovine serum albumin (BAPBS). After light centrifugation of the suspensions, 12 suckling mice were inoculated intracerebrally with 0.02 ml of each supernatant fluid. Brain tissue from sick or paralysed mice was used as a source of virus for passage.

Cell cultures. Suspensions of suckling-mouse brain (10%) were prepared in BAPBS and clarified at 1500 *g* for 10 min. Three Vero cell culture tubes were inoculated with 0.1 ml of the brain supernatant, incubated at 37°C, and examined daily for cytopathic effect (CPE). Cultures were maintained on Eagle's minimal essential medium containing 2 ml of heated fetal bovine serum per 100 ml. When CPE appeared, supernatant fluids were harvested and stored at —70°C. Cells were scraped from the tubes, resuspended in 0.2 ml of maintenance medium, and deposited in small drops on polytetrafluoroethylene-coated microscope slides with either 8 or 12 wells. Slides were allowed to dry in air, were fixed in acetone for 10 min, and were then stored at —70°C until used as antigens in the indirect fluorescent antibody (IFA) test.

Immunological characterization

Virus strains and antisera. Two human strains of Lassa virus were employed: L. P., isolated in 1969 from a case that originated in Nigeria; and T. J., recovered in 1976 from a patient who became infected in Sierra Leone. Each strain had undergone less than 10 passages in mice or cell cultures. The Armstrong strain of lymphocytic choriomeningitis (LCM) virus was also used. Antisera were prepared by inoculating infected cell culture fluids intraperitoneally into guinea-pigs or hamsters. Booster inoculations of virus were given in some instances. Immune sera were prepared in guinea-pigs for the L.P. strain and the S.B. strain of Lassa virus; the latter originated from a patient with Lassa fever in Sierra Leone in 1972. Immune ascitic fluid to

[1] Research Microbiologist, Special Pathogens Branch, Center for Disease Control, Public Health Service, US Department of Health, Education, and Welfare, Atlanta, GA 30333, USA.
[2] Head, Arbovirus Unit, National Institute for Virology, Johannesburg, South Africa.
[3] Virologist, National Institute for Virology, Johannesburg, South Africa.
[4] Chief, Special Pathogens Branch, Center for Disease Control, Atlanta, GA 30333, USA.

Mozambique strain AN 20410 was raised in mice by three intraperitoneal inoculations of 0.2 ml of 20% mouse brain suspension given at weekly intervals. Sarcoma 180 mouse cells were administered on day 22 and ascitic fluids were harvested 2 weeks later.

Complement fixation (CF) test. Antigens for CF testing were made from infected Vero cell cultures when they exhibited advanced CPE, usually 8–10 days after inoculation of about 1000 $TCID_{50}$ of virus. The resulting suspensions were centrifuged at low speed and the supernatant fluids, which comprised the antigen, were stored at —70°C. Control Vero cell antigens were prepared from normal cultures processed in a similar fashion. The CF test was carried out according to a standard procedure (*1*). Antigens and antisera were diluted in twofold steps beginning at a concentration of 1:4.

Indirect fluorescent antibody test. This procedure was carried out as previously described (*2*). Fluorescein isothiocyanate-conjugated immunoglobulin G reagents against human, guinea-pig, hamster, and mouse were obtained commercially. Evans blue at a final concentration of 1:2000 was used as counterstain. Specificity of conjugates and reference antisera was established by tests on uninfected Vero cells and by quantitative reactions with homologous as well as closely related (Lassa) and distantly related (LCM and Tacaribe) arenaviruses.

RESULTS

Virus isolation

Tissue suspensions from 210 rodents (*3*) were inoculated into suckling mice. Five of 112 *M. natalensis* yielded agents that produced illness, paralysis, and death in mice. Isolates were obtained between the 10th and 14th days after inoculation from 4 of 64 rodents captured in and near a paddy field, but from none of the 35 captured in the village of Mopeia itself. The infected suspensions were poorly pathogenic, as shown by the fact that less than half of each inoculated mouse litter became sick. After passage in suckling mice, the incubation period decreased to 8 or 9 days and the mortality rate increased, but some mice still survived. Lyophilized preparations of strain AN 20410 yielded a titre of 10^4 measured intracerebrally in suckling mice. Inoculation of virus intracerebrally or intraperitoneally into 4-week-old mice produced no apparent ill effects.

Infectivity of the virus with a titre for suckling mice of 10^4 was destroyed by exposure to 50% chloroform for 10 min at room temperature.

Virus characterization

Mouse-brain suspensions from the five isolates were inoculated into Vero cell cultures. Cytopathic changes typical for arenaviruses appeared within 4–6 days. Individual cells became spindle shaped and appeared refractile. Affected cells became detached from the cell sheet, forming progressively larger holes in the monolayer. Before CPE was far advanced, cells were scraped from the tube walls and tested for Lassa virus antigen by IFA. Most cells showed brightly stained intracytoplasmic inclusions as well as distinct peripheral staining features characteristic of Lassa virus.

Immunological studies

The five virus strains were examined in CF and IFA tests with mouse ascitic fluid prepared against AN 20410 virus. Each strain reacted to titres of 1:32–1:64 by both techniques. AN 20410 virus was next compared by the CF technique with Lassa virus

Table 1. Relationship between Lassa and Mozambique AN 20410 viruses by complement fixing reaction

Antigen	Reciprocal titre in:									
	Human convalescent sera					Animal immune sera and ascitic fluid				
	L.P.	A.M.	B.O.	M.S.	V.Y.	L.P. guinea-pig	S.B. guinea-pig [a]	AN 20410 hamster [a]	AN 20410 hamster	AN 20410 MAF [b]
L.P.	32	16	64	64	128	⩾1024	⩾1024	128	32	8
T.J.	16	16	64	128	256	512	1024	64	32	<8
AN 20410	8	8	8	16	32	256	512	512	128	32

[a] Animals received two injections.
[b] Mouse ascitic fluid.

Table 2. Indirect immunofluorescent antibody reactions between Lassa and Mozambique AN 20410 viruses

Antigen	Reciprocal titre in:									
	Human convalescent sera					Animal immune sera and ascitic fluid				
	L.P.	B.M.	B.O.	M.S.	V.Y.	L.P. guinea-pig	S.B. guinea-pig [a]	AN 20410 hamster [a]	AN 20410 hamster	AN 20410 MAF [b]
L.P.	64	32	128	128	256	256	256	256	64	32
AN 20410	16	8	64	32	64	128	128	256	256	128

[a] Animals received two injections.

[b] Mouse ascitic fluid.

strains isolated in Nigeria and Sierra Leone. The results given in Table 1 show small but reproducible differences between the Mozambique and Lassa agents when animal antisera were used, as well as a 2- to 4-fold reduction in titre when AN 20410 antigen was reacted with sera from West Africans convalescent from Lassa fever. The human sera had been obtained 3 weeks to 6 months after the onset of symptoms. The two Lassa virus antigens were identical. In contrast to a previous report (*4*), no differences in CF titre were found in sera from Nigeria, Sierra Leone, and Liberia. Similar relationships were observed with IFA test results (Table 2), although differences between Lassa virus and the Mozambique virus were less marked when sera from animals that received more than a single virus inoculation were employed. The Armstrong strain of LCM virus gave weak or no reaction at 1:4 dilution of animal sera for Lassa and Mozambique agents. LCM guinea-pig serum with a homologous titre of 1:1024 stained cells infected with both Lassa and Mozambique viruses to a dilution of 1:16.

Nineteen sera from *M. natalensis* captured during the field studies were available for antibody studies. Although an anti-*Mastomys* globulin conjugate was not available, it was found that an anti-*Rattus* conjugate gave a satisfactory specific reaction when used to measure IFA antibodies in *Mastomys* experimentally infected with Lassa virus. Four of the 19 sera had antibodies to the AN 20410 strain in titres of 1:8-1:32. These four also reacted with the L.P. strain of Lassa virus, but three of them had 2- to 4-fold lower titres to that antigen.

Sera from nine persons who had worked with the original isolates under ordinary laboratory conditions were also tested for IFA antibodies to both AN 20410 and L.P. viral antigens, with negative results.

DISCUSSION

Lassa virus, the etiological agent of a severe, acute systemic disease with frequent haemorrhagic manifestations, has so far been isolated from man and *M. natalensis* only in the West African countries of Nigeria, Liberia, and Sierra Leone (*4*, *5*, *6*, *7*, *8*).

Because Lassa virus, like most other arenaviruses, appears to be a single-host rodent parasite, and because *M. natalensis* is widely distributed over the southern two-thirds of the African continent (*9*), the presence of Lassa virus or a closely related arenavirus in south-east Africa does not represent a biological surprise. The isolations do, however, raise several questions of more than academic interest. Is this virus a minor antigenic variant of Lassa virus or is it a new arenavirus immunotype? Although the present serological results are inadequate to permit a definite answer, we suspect that the agent from Mozambique is a new arenavirus. It produced illness and death in suckling mice, a phenomenon not previously noted for Lassa virus; it has so far failed to cause any recognized illness in man; and it was found more than 2500 miles from the place in which Lassa virus is known to be endemic. Similar biological diversity has been documented in the case of some South American arenaviruses (*10*). Definitive identification of these new strains awaits results of neutralization tests or their equivalent to measure type-specific virion surface antigens. The problem is complicated by failure so far to obtain neutralization of Lassa virus plaques with hyperimmune animal or convalescent human sera.

It is of special importance to determine whether the new strains are pathogenic for man and whether the *M. natalensis* of West Africa and south-east Africa are truly identical. The answer to the first

question depends on the chance recognition of Lassa fever outside West Africa or the elucidation of pathogenic properties of viruses from both locations in animals such as nonhuman primates. As to *Mastomys*, there is already significant evidence that geographic and perhaps ecological races or subspecies occur in Africa (*11*, *12*). Systematic mapping of *Mastomys* as well as its arenavirus parasites deserves serious international attention. This need will become urgent if the Mozambique virus is shown to be a new arenavirus which is not virulent for man.

RÉSUMÉ

ISOLEMENT À PARTIR DE *MASTOMYS NATALENSIS*, EN AFRIQUE DU SUD-EST, D'UN ARÉNAVIRUS TRÈS PROCHE DU VIRUS DE LASSA

Cinq souches de virus immunologiquement identiques ont été isolées à partir du rongeur *Mastomys natalensis* au cours d'études sur les infections à arbovirus au Mozambique. Ces agents, inoculés à des souris blanches à la mammelle, entraînaient une infection fatale; cependant la période d'incubation était de 8 à 17 jours et une mortalité sporadique seulement s'observait après inoculation primaire de suspensions de tissu de *Mastomys*. Ces agents se multipliaient en cultures de la lignée cellulaire Vero et produisaient des corps d'inclusions cytoplasmiques semblables à ceux qui caractérisent le virus de Lassa à l'examen par la technique d'immunofluorescence indirecte. Des épreuves réciproques de fixation du complément et d'immunofluorescence ont révélé des différences quantitatives faibles, mais reproductibles, entre le virus du Mozambique et le virus de Lassa. Le virus du Mozambique n'était pas apparenté antigéniquement au virus de la chorioméningite lymphocytaire, autre arénavirus qui a des antigènes en commun avec le virus de Lassa.

Sur 19 sérums de *Mastomys* capturés dans la région étudiée, 4 se sont révélés, par immunofluorescence, posséder des anticorps à l'égard des virus du Mozambique et de celui de Lassa. Les sérums des membres du personnel qui avaient travaillé sur ces nouveaux agents étaient négatifs. Bien que, pour des raisons techniques, on n'ait pas obtenu de résultats nets de la neutralisation du virus, il semble bien d'après les données dont on dispose que les souches de virus du Mozambique ne sont pas identiques à celles du virus de Lassa d'Afrique occidentale. Il reste à déterminer la pathogénicité des arénavirus de l'Afrique du sud-est pour l'homme et d'autres primates.

REFERENCES

1. CASEY, H. L. Standardized complement fixation method and adaptation to micro rest. Washington, DC, US Department of Health, Education, and Welfare, *Public health monograph*, No. 74, 1965.
2. WULFF, H. & LANGE, J. W. Indirect immunofluorescence for thediagnosis of Lassa fever infection. *Bulletin of the World Health Organization*, **52**: 429-435 (1975).
3. MCINTOSH, B. M. ET AL. *Culex (Eumelanomyia) rubinotus* Theobald as vector of Banzi, Germiston and Witwatersrand viruses. II. Infections in sentinel hamsters and wild rodents. *Journal of medical entomology*, **12**: 641-646 (1976).
4. MONATH, T. P. ET AL. Lassa fever in the Eastern province of Sierra Leone, 1970-1972. II. Clinical observations and virological studies on selected hospital cases. *American journal of tropical medicine and hygiene*, **23**: 1140-1149 (1974).
5. BUCKLEY, S. M. & CASALS, J. Lassa fever, a new virus disease of man from West Africa. *American journal of tropical medicine and hygiene*, **19**: 680-691 (1970).
6. WULFF, H. ET AL. Recent isolations of Lassa virus from Nigerian rodents. *Bulletin of the World Health Organization*, **52**: 609-612 (1975).
7. MONATH, T. P. ET AL. A hospital epidemic of Lassa fever in Zorzor, Liberia, March–April 1972. *American journal of tropical medicine and hygiene*, **22**: 773-779 (1973).
8. MONATH, T. P. ET AL. Lassa virus isolation from *Mastomys natalensis* rodents during an epidemic in Sierra Leone. *Science*, **185**: 263-265 (1974).
9. ISAÄCSON, M. The ecology of *Praomys (Mastomys) natalensis* in Southern Africa. *Bulletin of the World Health Organization*, **52**: 629-635 (1975).
10. WEBB, P. A. ET AL. Behavior of Machupo and Latino viruses in *Calomys callosus* from two geographic areas of Bolivia. In: Lehmann-Grube, F., ed. *Lymphocytic choriomeningitis virus and other arenaviruses*. Berlin, Heidelberg, New York, Springer-Verlag, 1973, pp. 313-322.
11. COETZEE, C. G. The biology, behaviour, and ecology of *Mastomys natalensis* in Southern Africa. *Bulletin of the World Health Organization*, **52**: 637-644 (1975).
12. BELLIER, L. The genus *Mastomys* in the Ivory Coast. *Bulletin of the World Health Organization*, **52**: 665 (1975).